Viral Clearance/Adventitious Agent Testing/Column Reuse

Date: September 5, 2001
From: Rona LeBlanc, Ph.D., DTP
To: BLA STN# 125029 file
Through: Barry Cherney, Ph.D., Deputy Director, DTP;
Amy Rosenberg, M.D., Director, DTP
Product: Drotrecogin alfa (activated protein C, recombinant, human, human ----------)
Proposed Use: Treatment of sepsis associated with acute organ failure (severe sepsis)
Sponsor: Eli Lilly and Company

Note: Drs. Gibbes Johnson and Fred Mills did the remainder of the CMC/Product review. Please see their reviews for more information.

Table of Contents for Drug Substance:

4. ----------
5. ----------

C. ----------
1. ----------
2. ----------
3. ----------
4. ----------
5. ----------

D. ----------
1. ----------
2. ----------
3. ----------
4. ----------
5. ----------

E. ----------
1. ----------
2. ----------
3. ----------
4. ----------
5. ----------

F. ----------
1. ----------
2. ----------
3. ----------

G. ----------

H. ----------
1. ----------
2. ----------

VI. Action Items

I. Rationale and Background:

Recombinant human Activated Protein C (rhAPC), produced in human ----------, is a recombinant version of the natural human plasma-derived activated protein C, differing in the ----------. APC is a serine protease activated from the zymogen Protein C. RhAPC has antithrombotic, pro-fibrinolytic and anti-inflammatory activities. Eli Lilly and Company is developing rhAPC as a therapy for patients with severe sepsis.

The Protein C pathway regulates the inhibition of blood coagulation by limiting thrombin formation resulting in decreased fibrin deposition. The ability of APC to limit thrombin formation through inactivation of Factor Va and Factor VIIIa is the basis for APCs antithrombotic function.

Plasma-derived APC has been shown to have profibrinolytic effects thought to result indirectly from APC's effect on two components of the fibrinolytic

pathway (inhibition of plasminogen activator inhibitor-1 (PAI-1) and inhibition of activation of thrombin activatable fibrinolysis inhibitor (TAFI; also referred to as procarboxypetidase B). APC is thought to indirectly inhibit activation of TAFI by virtue of its ability to inhibit thrombin generation.

Thrombin has been shown to be a potent mediator of several inflammatory processes. Thrombin upregulates the expression of P-selectin on endothelial cells, facilitating neutrophil adhesion and activation. Thrombin itself is chemotactic for neutrophils, and also causes the expression of platelet activating factor, a potent neutrophil activator (Esmon 1998). Through its antithrombotic properties, rhAPC may indirectly mitigate these inflammatory pathways by inhibiting the generation of thrombin. Several in vitro studies suggest that rhAPC may also interfere directly with inflammatory pathways in mechanisms both dependent and independent of its enzymatic activities.

Another possible mechanism for rhAPC's role in the reduction of the inflammatory response is suggested in studies performed by Grinnell et al. 1994. In an effect independent of its serine protease activity, plasma-derived APC or recombinant human Protein C inhibited in vitro E-selectin-mediated cell adhesion, an event critical for the recruitment of neutrophils following an inflammatory response.

Recombinant human Activated Protein C is intended for use as a therapeutic for patients with severe sepsis defined as sepsis associated with organ dysfunction which has developed in the context of infection.

Recent evidence indicates that the systemic response to infection includes not only an inflammatory component, but also a procoagulant response. Pro-inflammatory cytokines, including TNF-α?IL-1, and IL-6, are capable of activating coagulation and inhibiting fibrinolysis, while the procoagulant thrombin is capable of stimulating multiple inflammatory pathways. As sepsis progresses, the imbalance between coagulation and fibrinolysis manifests as coagulopathy and microvascular thrombosis and may ultimately be a primary factor in the development of multiple organ failure. Reported mortality rates for patients with severe sepsis range between 30% and 60%. Activated Protein C, with its known mechanisms of action, may play a key role in modulating the intensive hemostasis and inflammatory host response to infection. Current therapy for severe sepsis includes appropriate antimicrobial treatment, source control of the infection (eg, surgical debridment of necrotic tissue), volume resuscitation, cardiopulmonary support and support for other failing organs. However, no therapy specifically targets the inflammatory and procoagulant host response to the infection that results in the development of multiple organ dysfunction.

The sponsor claims that rhAPC would be the first specific compound shown to reduce 28-day all-cause mortality in patients with severe sepsis.

II. Characterization of the Cell Substrate for ----------production

A. Origin and History of the Parental Cell Line

The cDNA used for the generation of the production cell line for Lilly recombinant human Protein C (----------) was derived from the ----------. The human ---parent cell line, into which the Protein C expression vectors were introduced, is a ---------- line of primary human ----------. The cell line was isolated in ----------. An analysis by cloning and sequencing of the cellular-viral junctions has revealed that the ---------- cell line contains the fragment ----------, or approximately ---------- This ----------.

B. Characterization of the Master Cell Bank (MCB) & Description of Test Methods

---- different sets of Master Cell Banks (MCB) and Working Cell Banks (WCB) have been prepared, the ----- set (---------- MCB and ---------- WCB) in 1991 and the ---------set (----------MCB and ----------, Lot ----------, WCB) in 1998. Only the ----------set of cell banks will be used in the manufacturing of rhAPC; they were also used for the latter part of the Phase III clinical trial campaign. The ---------- MCB and WCB were prepared from a ---------- of the first WCB. The first WCB was chosen for ---------- rather than the first MCB, as the WCB had already been adapted to ----------culture without the need for ----------. The additional subcloning was done to further increase the assurance that the cell line used in the late Phase III clinical trials and for production was derived from ----------. Both sets of banks were extensively tested for the presence of adventitious agents and characterization of the expression construct. See the tables that follow in this section.

The tests performed on ---------- MCB (the ----------MCB) are listed below. An explanation of the methods follows.

Taken from Table I.C.6 on pages --------- (identical to Table I.D.60, pages 587-590).

Test	**Method**	**Results**
Identity	----------	Enzymes were consistent with cells of human origin. Also human karyology observed.
Retroviruses, adventitious agents	----------	No virus or virus-like particles seen
----------	----------	----------
----------	----------	----------

Retroviruses, ---------- ----------	----------	Negative
	----------	---------- ----------
	----	----------
Sterility	----------	Negative
Mycoplasma	---------- ----------	Negative
Adventitious agents, ---------- ----------	----------	----------
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Commercial MCB: The tests performed on ----------, MCB are listed below. An explanation of the methods follows.
Taken from Table I.C.8 on page 314 (identical to Table I.D.64, pages 594).

Test	**Method**	**Results**
Identity	----------	Consistent with cells of human origin.
Bacterial/Fungal Contamination	----------	Negative
Mycoplasma	---------- ----------	Negative
Retroviruses, adventitious agents	----	No identifiable retroviral-like particle
Adventitious agents, ---------- ----------	----------	Negative
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Comments: Appropriate testing was done on the MCB as per ICH Q5A. This provides added assurance of safety and reproducibility. The MCB was of human origin and was free of endogenous and adventitious agents. Thus, the characterization of the MCB is acceptable.

Test Methods

Cell Line Identification and Characterization (----------)

----------: Description of Cells and Detection of Viruses and Retroviruses
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A. Characterization of the Working Cell Bank (WCB) ----------

The tests performed and the results are listed below. Test methods were the same as those used above for the MCB.
Taken from Table I.C.6 on pages -------- (identical to Table I.D.60, pages 587-590).

Test	**Method**	**Results**
Identity	----------	Enzymes were consistent with cells of human origin. Also human karyology observed.
Retroviruses, adventitious agents	-------	No virus or virus-like particles seen
Retroviruses, ----------	----------	Negative
	----------	----------.
Sterility (Bacterial/Fungal Contaminants)	----------	Negative
Mycoplasma	----------	Negative
Adventitious agents, ----------	----------	Negative
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Taken from Table I.C.8 on page 314 (identical to Table I.D.64, pages 594). WCB ----------

Test	**Method**	**Results**

Identity	---------- ----------	Consistent with cells of human origin.
Bacterial/Fungal Contamination	----------	Negative
Mycoplasma	----------	Negative
Retroviruses, adventitious agents	----	No identifiable retroviral-like particle
Adventitious agents, ----------	----------	Negative
	----------	Negative
Retroviruses	-----	----------

Comments: The WCB was appropriately tested as per ICH Q5A. This provides added assurance of safety and reproducibility. It is of human origin and is free of adventitious agents. Thus, characterization of the WCB is acceptable.

D. ----------

Test **Method** **Results**

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III. Adventitious Agent Screening During Manufacture

A. Animal-derived Raw Materials

1. --------------------------- was obtained from BSE-free herds in the United States or New Zealand. This is used in preparation of the MCB, WCB and consistency lots. The Certificates of Analysis are located on pages 138 and 188. Bovine virus testing performed was per 9CFR 113.53 and included -- Tests for --------------------------------- were also done.
2. Bovine ---------was obtained from animals in the United States and is used in production of the consistency lots as well as in routine manufacture. The Certificate of Analysis is located on page 178. Bovine virus testing performed was per 9CFR 113.53. Tests for --------------------------- were also done.
3. Bovine -------------- was obtained from herds in the United States or New Zealand. It is used in the --- -----------. The Certificate of Analysis is located on page 227. Bovine virus testing performed was per 9CFR 113.53. Tests for ------------------------------ --------- were also done.

The following is a description of the assays that were used to test and characterize the animal sourced raw materials (Pages 655-656, section I.D.3). Copies of the actual reports are provided in Section I.I.1, Viral Safety Reports.

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Comments: The raw materials of animal origin were appropriately tested for viral contamination.

B. Drug Substance Consistency Run (unprocessed bulk; bioreactor harvest)
Unprocessed bioreactor solutions are obtained at the end of a production run from each ----------liter bioreactor. Lots tested were ----------.

Test	**Method**	**Acceptance Limits**
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The following is a description of the assays that were used to test unprocessed bulk harvest at the end of bioreactor run (Pages 656-657, section I.D.3). Copies of the actual reports are provided in Section I.I.1, Viral Safety Reports.

Comments: The harvest materials have been appropriately tested for adventitious agents. The routine in-process testing scheduled during manufacture should be sufficient to detect most potential contaminants.

IV. Summary of Adventitious Agent Testing in Cell Banks and During Manufacture

A. The characterization of the MCB, WCB, and ------ is acceptable and provides added assurance of safety.

B. The animal-derived raw materials were tested as per 9 CFR 113 to provide added assurance of safety. The tests were acceptable.

C. --------consistency lots were tested for adventitious agents and the routine in-process testing should be sufficient to detect most potential contaminants.

V. Viral Clearance Studies

Studies to measure both the robustness and clearance of retroviruses in particular were done using a scaled-down process using the model viruses listed below. Both new and used column resins were tested. The ---------- ---------- is used once and the cutoff size is approximately --- nm.

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1. ----------

	1st run	2nd run
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---------- ----------	----------	----------
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2. ----------

Comments: ----------

	1st run	2nd run
----------	----------	----------
----------	----------	----------

----------: ----------

G. Summary of Virus Clearance:

------ independent studies were done for all the steps involved (----------). Both new and used column resins were tested and show similar clearance values. The ---------- ---------- can be used ---- times, and the ----- resin can be used --- times. The clearance studies were done at ---------- and at Lilly. The sponsor used --model viruses covering different physico-chemical properties (----------). Indicator

cell lines and infectivity assays were appropriate for the viruses tested and were done in replicates. Cytotoxicity and interference assays were done, as were inactivation studies ----------. Also, the sponsor stated the mechanism of clearance (inactivation vs. removal) for some of the steps. In addition, ----------

Cumulative log $_{10}$ reduction values based on a worst case scenario ranged from approximately ---------- Log $_{10}$ for all viruses tested, including small viruses (----------).

Overall, the assays used were acceptable and viral clearance by the columns, single-use ---------- was sufficiently robust to increase assurance of freedom from adventitious agents.

H. ---------- Solution Inactivation Studies

1. ----------

Virus	Titer T=-------	Log$_{10}$ Reduction T=-------	T=-------	T=-------	T=---------
---	---	---	---	---	---
---	---	---	---	---	---
---	---	---	---	---	---
---	---	---	---	---	---
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Virus	Titer T=-------	Log_{10} Reduction T=-------	T=-------
---------	--------- ----------	--------- ----------	--------- ----------
---------	--------- ----------	--------- ----------	--------- ----------
---------	---------	---------	---------
---------	---------	---------	---------
---------	---------	---------	---------

Comments: The viruses (----------) were reduced by at least -- logs on contact with the solution. ------- was reduced by at least-- logs within 15 minutes. The cleaning solution used would help to reduce carryover of viruses to the next batch, and reduce potential virus in downstream processing. Virus remaining on the columns should be inactivated prior to column reuse.

***Note:** In the summary tables, the sponsor incorrectly averaged the reduction values of all time points for a given virus per experiment. Then, the values of both experiments per virus were averaged. That method of calculation does not give a clear picture of inactivation over time. An inactivation curve is needed. Thus, I went to the study reports for the actual results per time point per experiment.

2. [

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Virus	Titer T=----------	Log_{10} Reduction T=---------	T=--------	T=-------	T=----------
----------	----------	----------	----------	----------	----------

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---	---	---	---	---	---
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Second Experiment:

Virus	Titer T=---	Log_{10} Reduction T=---	T=---
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---	---	---	---
---	---	---	---
---	---	---	---

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Comments: The viruses tested (----------) were reduced by over -- logs upon contact with the solution. --- was reduced by at least -- logs within 15 minutes. The cleaning solution used would help to reduce carryover of viruses to the next batch, and reduce potential virus in downstream processing. Virus remaining on the columns should be inactivated prior to column reuse.

***Note:** In the summary tables, the sponsor incorrectly averaged the reduction values of all time points for a given virus per experiment. Then, the values of both experiments per virus were averaged. That method of calculation does not give a clear picture of inactivation over time. An inactivation curve is needed. Thus, I went to the study reports for the actual results per time point per experiment.

VI. Action Items/Comments to Sponsor

None.